Investigating chemical changes during shelf-life of thermal and high pressure high temperature sterilized carrot purees: a '*fingerprinting kinetics*' approach

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Abstract

This work investigates chemical changes during shelf-life of thermal and high pressure high temperature (HPHT) sterilized carrot purees using a "*fingerprinting kinetics*" approach. Fingerprinting enabled selection of Strecker aldehydes, terpenes, phenylpropanoids, fatty acid derivatives and carotenoid degradation products as volatiles clearly changing during shelf-life. Next, kinetic modelling of these volatiles was performed to compare their reaction kinetics during storage in differently sterilized samples. Immediately after processing, the Strecker aldehydes were detected at higher levels in thermal sterilized samples. During storage, the compounds increased at a comparable rate in thermal and HPHT processed samples. In contrast, immediately after processing, most of the naturally occurring terpenes and phenylpropanoids were better preserved in HPHT treated samples. Nevertheless, by the end of storage, the concentration of these compounds decreased to almost the same level in both thermal and HPHT sterilized samples (with a higher degradation rate in HPHT sterilized samples).

Keywords

Thermal processing; high pressure high temperature processing; shelf-life; chemical reactions; carrot puree; headspace GC-MS fingerprinting; kinetic modelling.

Abbreviated running title

Investigating chemical changes in sterilized carrot during storage

1. INTRODUCTION

In previous studies, the quality impact of thermal and high pressure high temperature (HPHT) processing was compared immediately after processing (Kebede et al., 2013; Kebede et al., 2014a; Kebede et al., 2014b). Aiming for a fair comparison, the processing conditions of both treatments were selected targeting at an equivalent microbial inactivation (F_0 = 5 min). In these studies, HPHT processing enforced a clear different effect on chemical reactions compared to thermal processing. For instance, Strecker aldehydes (e.g., 3-methylbutanal, 2-methylbutanal and 2-methylpropanal) were detected in a significantly higher amount in thermally sterilized vegetables compared to HPHT sterilized vegetables. Furthermore, HPHT processing, in comparison to thermal processing, enhanced the formation of oxidative-degradation reaction products of mainly terpenes, free fatty acids, phenylpropanoids and carotenoids. However, since the quality of sterilized foods further changes during shelf-life, it is unknown whether these differences will remain the same or changes during storage. Considering consumer expectations, quality should be maintained at a targeted level during the period between processing and purchase as well as between purchase and consumption. There is a need for studies which not only take into account quality changes during preservation, but also as a function of shelf-life (Kilcast, 2000; Arnoldi, 2001; Awuah, Ramaswamy, & Economides, 2007; van Boekel et al., 2010).

In this work, chemical changes during shelf-life of thermal and HPHT sterilized vegetable purees were investigated. Through a comprehensive integration of high throughput analytical instruments (GC-MS), advanced data analysis techniques (multivariate data analysis (MVDA)) and kinetic models, a '*fingerprinting kinetics*' approach was developed (Grauwet, Vervoort, Colle, Van Loey, & Hendrickx, 2014). This newly integrated approach is used as a central research strategy for investigating chemical changes during shelf-life. As a case study, a carrot

puree was chosen. Unfortunately, today, it is not possible to fingerprint the whole chemical composition of a food matrix using one particular analytical method (e.g., GC-MS). In this work, the volatile food fraction was selected to start the investigation. Volatiles are often linked to process-induced reactions. Being regularly degradation products of major food components (e.g., sugar, fat, nutrients), they can be approached as witnesses for what is happening in a complex food system. Therefore, the volatile fraction of processed and stored carrot purees was analyzed with a headspace solid-phase microextraction GC-MS (HS-SPME-GC-MS) procedure. The HS-SPME-GC-MS procedure was optimized targeting as many compounds as possible of the analyzed volatile extract and thus, the procedure can be considered as an 'untargeted' approach (**Fig. 1**). The obtained GC-MS data was analyzed at two levels. At the first level, with the aid of MVDA, fingerprinting was used for selecting volatiles and reactions significantly changing during shelf-life in both thermal and HPHT sterilized carrot purees. At the second level, based on the data from the fingerprinting as a function of time, kinetic modelling of the selected volatiles was performed to compare their reaction kinetics during storage in thermal and HPHT sterilized carrot purees (**Fig. 1)**.

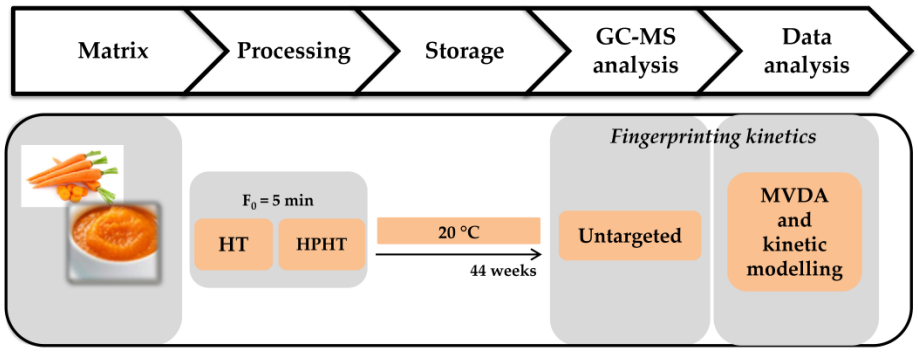


Fig. 1. Schematic overview of the experimental approach of the present work, for investigating chemical changes during shelf-life of thermal (HT) and high pressure high temperature (HPHT) sterilized carrot purees. The type of matrix used, the applied processing techniques, storage condition, headspace solid-phase microextraction GC-MS (HS-SPME-GC-MS) analysis and data analysis is described. A '*fingerprinting kinetics*' approach was used for evaluating shelf-life changes. At the first level, with the aid of multivariate data analysis (MVDA), fingerprinting was used for selecting volatiles and reactions significantly changing during shelf-life. At the second level, kinetic modelling of the selected volatiles was performed to compare their reaction kinetics during storage in thermal and HPHT sterilized carrot purees.

2. MATERIALS AND METHODS

2.1. *Sample preparation*

A single batch of freshly harvested carrots (cv. *Nerac*) was purchased at a local market. The carrots were carefully washed, peeled and cut into standardized cylindrical pieces of approximately 1 cm thickness. The carrot pieces were packed into low-density polyethylene bags. To prevent enzymatic reactions during processing and storage, the packaged carrots were blanched at 95 °C for 8 min in a water bath (Haake W15 DC-10, Clausthal-Zellerfeld, Germany). The blanching conditions were validated using a qualitative and quantitative peroxidase test (Adebooye, Vijayalakshmi, & Singh, 2008). After blanching, the plastic bags were immediately cooled in ice water for 10 min, frozen in liquid nitrogen and stored in a freezer at -40 °C until processing. Prior to processing, the samples were thawed overnight at 4 °C. In order to prepare the puree, deionized water was added to the blanched carrot (1/1 w/w), blended for 1 min using a Buchi mixer (B-400, BUCHI, Switzerland) and further homogenized by high pressure homogenization (at 1000 bar while temperature maintained < 4 °C) (Panda 2K, Gea Niro Soavi, Mechelen, Belgium).

2.2. Thermal and HPHT treatment

Aiming at objective comparison of sterilization impacts on the product, the processing conditions in both thermal and HPHT sterilization were selected targeting an equivalent inactivation of spores of *Clostridium botulinum* ($F_{121.1^0C}^{10^0C}$ (F_0) = 5 min). Due to the lack of reliable kinetic data as a result of incomplete understanding of the combined effect of pressure and temperature on inactivation of *Clostridium botulinum* spores (Van der Plancken et al., 2012), in the present work, the HPHT treatment was considered as pressure assisted thermal treatment. In other words, for calculating the F_0 value, only the integrated thermal effects derived from the time-

74 temperature profiles in the product were taken into account. For both treatments, plastic bags
75 (100 ml volume) were filled with 60 ± 0.5 g of purees and vacuum-sealed.

76 The thermal treatment was carried out in a static steriflow pilot retort (Barriquand, Paris,
77 France). The samples were treated at a process temperature of 121 °C during a holding time of
78 2.4 min. The coming up time of the retort was 8.5 min. Temperature profiles in the retort and at
79 the coldest point of the product were recorded using type T thermocouples (Ellab, Hillerød,
80 Denmark).
81 For HPHT treatment, a pilot-scale unit (2.5 L, 10 cm inner diameter, developed by Resato,
82 Solico, Unilever and Wageningen UR Food and Biobased Research, The Netherlands) was used,
83 in which pressure was built up at a rate of 30 MPa/s. During HPHT treatment, starting from
84 room temperature, using only compression heating, the product temperature cannot be raised to
85 the point where inactivation of spores under high pressure is feasible. Therefore, prior the actual
86 HPHT treatment, it is essential that the samples were preheated at atmospheric pressure to
87 experimentally determined initial temperature. The samples were preheated in a water bath for
88 10 min at 90 °C before the actual high pressure processing. When the desired initial temperature
89 was achieved, the samples were transferred to the high pressure vessel. The pressure in the
90 vessels was increased through direct compression. The pressure medium was water. Due to the
91 pressurization (at 700 MPa), the temperature inside the product was increased from 90 °C to
92 124.8 °C through compression heating. The pressure was held for 3 min. At the end of the
93 holding time, the pressure was released from the vessels, which was accompanied with
94 temperature drop inside the product (decompression cooling).
95 Following completion of thermal and HPHT treatments, samples were transferred to ice water to
96 further cool the product.

2.3. *Storage*

To avoid oxygen uptake through the package during storage, sterilized plastic bags were placed in glass bottles, which were flushed with nitrogen gas and tightly closed. Next, the sealed glass bottles were stored in an incubator, protected from light, at 20 °C up to 44 weeks (**Fig. 1**). At fixed points in time, plastic bags were sampled from the incubators. The vegetable puree was aseptically (next to a Bunsen burner) transferred to small-volume (10 ml) polyethylene terephthalate tubes with a polyethylene cap. One gram of sample was taken for microbial analysis. Thereafter, the tubes were frozen in liquid nitrogen, wrapped with aluminium foil and stored in freezer at -40 °C until GC-MS analysis.

2.4. Microbial analysis

Microbial analysis was performed to verify growth of mesophilic (aerobic) and thermophilic (aerobic and anaerobic) microorganisms. Plate count agar was prepared for aerobic (mesophilic and thermophilic) bacteria, whereas the presence of anaerobic thermophiles were analyzed using reinforced clostridial agar. Throughout, the investigated shelf-life time for both processing conditions, the microbial growth was below detection limit (results not shown).

2.5. *HS-SPME-GC-MS analysis*

Samples were thawed overnight in the cooling room (4 °C). 2.5 g thawed sample and 2.5 ml saturated NaCl solution were mixed in a 10 ml amber glass vial (10 ml, VWR International, Radnor, PA, USA). The vials were tightly closed using screw-caps with silicon septum seal (Grace, Columbia, MD), homogenized and transferred to the cooling tray of the autosampler which was maintained at 10 °C. Headspace fingerprinting was conducted on a GC system (6890N, Keysight Technologies, Diegem, Belgium) coupled to a mass selective detector (MSD) (5973N, Keysight Technologies, Diegem, Belgium) and equipped with a CombiPAL

autosampler (CTC analytics, Zwingen, Switzerland). Targeting detection of as many as possible volatiles in a particular food extract, an HS-SPME–GC–MS method of analysis was optimized beforehand. In the selected method, the samples were incubated at 40 °C for 20 min under agitation at 500 rpm. Next, extraction of the volatiles was performed using HS-SPME fiber coated with 30/50 lm divinylbenzene/carboxen/polydimethylsiloxane (DVB/ CAR/ PDMS) (StableFlex, Supelco, Bellefonte, PA) at 40 °C for 10 min. The SPME fiber was inserted into the heated (230 °C) GC injection port for 2 min to desorb the volatile compounds. Prior to extraction, the fibers were conditioned and regenerated according to the manufacturer's guidelines in the conditioning station of the autosampler. Injection of the samples to the GC column was performed in split (1/5) mode. Chromatographic separation was carried out on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 lm film thickness, Agilent Technologies, Santa Clara, CA) with helium as carrier gas at a constant flow of 1.3 mL/min. The GC oven temperature was programmed from a starting temperature of 40 °C, which was retained for 2 min, to 172 °C at 4 °C/min, then ramped to 300 °C at 30 °C/min and kept constant at 300 °C for 2 min before cooling back to 40 °C. The mass spectra were obtained by electron ionization (EI) mode at 70 eV with a scanning range of m/z 35-400 and a scanning speed of 3.8 scans per second. MS ion source and quadrupole temperatures were 230 °C and 150 °C, respectively.

To minimize the phenomenon of fiber degradation, a new fiber was used for each treatment condition (thermal and HPHT). Per treatment condition, during analysis, the samples were randomized as a function of storage time. Possible fiber degradation was carefully monitored by analysis of a reference sample (blanched carrot samples), every 10 injections. Per storage time, each sample was analyzed six times.

2.6. Data analysis

2.6.1. Data pre-processing

GC/MS has long been the method of choice for identifying volatile compounds in complex mixtures. This method can fail, however, when acquired spectra are "contaminated" with extraneous mass spectral peaks, as commonly arise from co-eluting compounds and ionization chamber contaminants. These extraneous peaks can pose a serious problem for automated identification methods where they can cause identifications to be missed by reducing the spectrum comparison factor below some pre-set identification threshold. Automated mass spectral deconvolution and identification system (AMDIS) is an integrated set of procedures for first extracting pure component spectra and related information from complex chromatograms and then using this information to determine whether the component can be identified as one of the compounds represented in a spectral reference library. The practical goal is to reduce the effort involved in identifying compounds by GC/MS while maintaining the high level of reliability associated with traditional analysis. The program first deconvolutes the GC/MS data file to find all of the separate components. Furthermore, the program can be configured to build a retention index calibration file, to use the retention index data along with the mass spectral data. Therefore, in this work, all chromatograms were analyzed with AMDIS (Version 2.66, 2008, National Institute of Standards and Technology, Gaithersburg, MD, USA). The deconvoluted spectra were then analyzed with mass profiler professional (MPP) (Version 12.0, 2012, Keysight Technologies, Diegem, Belgium) aiming filtering and peak alignment. In MPP, there are several filtering options that may be applied: e.g., filter by frequency, abundance. The parameters in which the entities are filtered need to be optimized depending on the data under consideration. Samples are aligned or grouped together if their retention times are within the specified tolerance window and the mass spectral similarity. In cases, where retention time locking is applied, a smaller retention time tolerance window can be used.

The potential of the integrated data pre-processing steps, AMDIS and MPP can be described as: (i) improved identification due to the removal of impurities and extraction of pure spectrums; (ii) automated, standardized and much more reproducible data analysis; (iii) time saving and (iv) a possibility to calculate retention index.

The MPP yielded a spreadsheet containing peak areas. Before the statistical data analysis, a manual verification of the obtained spreadsheet was performed.

2.6.2. Multivariate data analysis

The multivariate data were analyzed with a multivariate data analysis (MVDA) which was carried out in Solo (Version 6.5, 2011, Eigenvector Research, Wenatchee, WA, USA). All data were mean-centered and the variables were weighed by their standard deviation to give them equal variance. In a first step, principal component analysis (PCA) was conducted as an exploratory technique to evaluate each data set and to detect potential outliers. To study the evolution during storage, per processing condition, partial least squares (PLS) regression was performed, with the volatiles as *X*-variables and the storage time as *Y*-variable. To qualitatively investigate impact differences induced by storage based on the selected models, bi-plots were generated. Even though bi-plots also graphically illustrate the importance of each variable for the classification, it is not straightforward to quantitatively rank the component's relevance. As a quantitative measure for selection of volatile compounds clearly changing during storage, variable identification (VID) coefficients were calculated. These values correspond to the correlation coefficient between each original *X*-variable and predicted (by the selected PLS-model) *Y*-variable. In this work, variables with an absolute VID value higher than 0.700 were considered to be important. These variables were identified and linked to possible reaction pathways. Tentative identification of the compounds was performed by comparing the deconvoluted mass spectrum with the reference mass spectra from both NIST spectral library

(NIST08, version 2.0, National Institute of Standards and Technology, Gaithersburg, MD, USA) and WILEY mass spectral data (Wiley2010, version 9, Hoboken, New York, USA). For identification, a threshold match of 90 % was taken into account. For further confirmation, visual inspection of spectral matching between the detected compound and the match from the library was performed.

2.6.3. Kinetic modelling and parameter estimation

Kinetic modelling was performed based on the rate equation of a degradation reaction. For a detailed discussion on the general principles of kinetic modelling, the reader is referred to the work of van Boekel (2008). The general rate equation of an n^{th} order reaction is expressed as **Equation 1**:

$$r = -\frac{dC}{dt} = kC^n \quad (1)$$

Where *r* is representing the rate of the reaction, *C* the concentration of the compound at time *t*, *k* the reaction rate constant and *n* the order of the reaction. **Equation 1** can be integrated with respect to time to obtain the course of concentration as a function of time. For a degradation reaction, integration of **Equation 1** for n = 0 and 1 (for zero- and first-order kinetics) leads to **Equation 2 and 3**, respectively:

$$\text{n} = 0 \quad : \quad C = C_0 - kt \quad (2)$$

$$\text{n} = 1 \quad : \quad C = C_0 . exp(-kt) \quad (3)$$

Zero- and first-order reactions are frequently reported for quality changes in foods. In the present work, for kinetic modelling of volatiles that were changing during storage, firstly, a suitable kinetic model was selected by visual inspection of different concentration plots. The selected models were evaluated based on visual inspection of the parity and residual plots and by the calculation of $R^2_{adjusted}$ (**Equation 4**). Next, the corresponding kinetic parameters were estimated using nonlinear regression (SAS version 9.4, Cary, USA).

$$R^2_{adjusted} = 1 - \frac{\left[(\mathrm{DF_{tot}}-1)\left(1-\frac{\mathrm{SS_{model}}}{\mathrm{SS_{total}}}\right)\right]}{\mathrm{DF_{error}}} \quad (4)$$

In **Equation 4**, where DF_{tot} and DF_{error} are degree of freedom of total and error, respectively and SS is the sum of squares

3. RESULTS AND DISCUSSION

At first, the headspace solid-phase microextraction GC-MS (HS-SPME-GC-MS) fingerprinting of time-related changes during shelf-life, identification of important shelf-life volatiles and linkage to reaction pathways will be discussed. Next, the reaction kinetics of the changes of the volatiles (selected by fingerprinting) will be determined.

3.1. Headspace SPME-GC-MS fingerprinting

3.1.1. Qualitative investigation of time-related changes during shelf-life

Fig. 2. depicts an exemplary GC-MS total ion chromatogram of the headspace of thermal and HPHT treated carrot purees at the start of storage (day 0). As described in section 2.6.1, the complex GC–MS data files were analyzed with a sequence of data pre-processing techniques (i.e., automated mass spectral deconvolution and identification system, AMDIS, and mass profiler professional, MPP). MPP yielded a spreadsheet containing peak areas, which was used as an input for the multivariate data analysis (MVDA).

For MVDA, after exploring the data with PCA (results not shown), PLS regression was conducted with the carrot headspace components considered as *X*-variables and storage time as continuous *Y*-variable. Per processing condition, the first two LVs explained a considerable amount of the *Y*-variance (96 % and 98 % for thermal and HPHT, respectively), with a large portion already covered by the first LV (**Fig. 3)**. Accordingly, for each processing conditions, a multivariate PLS model based on two LVs was selected. As can be seen from **Fig. 3**, the trend of

storage time can be clearly observed on the bi-plots. The first clear trend is the horizontal projection of the volatile fractions of the carrot purees from the left of the bi-plot to the right of the bi-plot. This dominant change during storage is described by the first LV, as indicated in the respective axis (at least 77 % Y-variance explained).

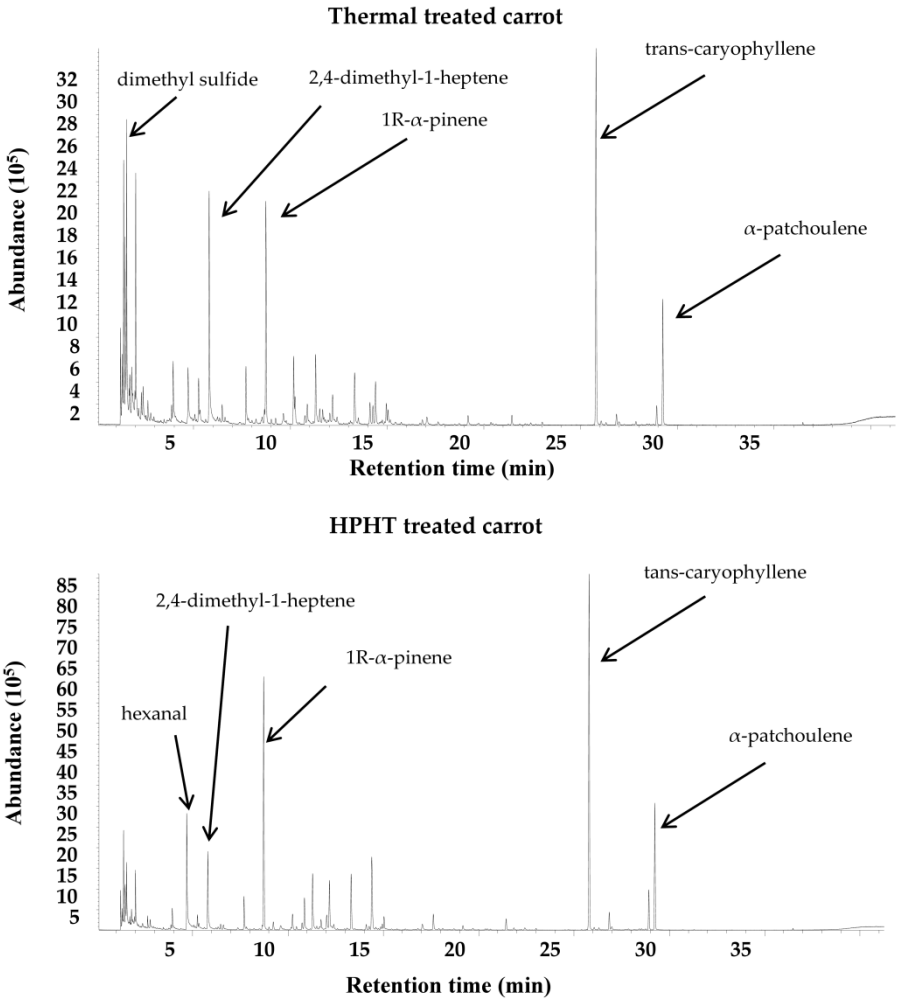
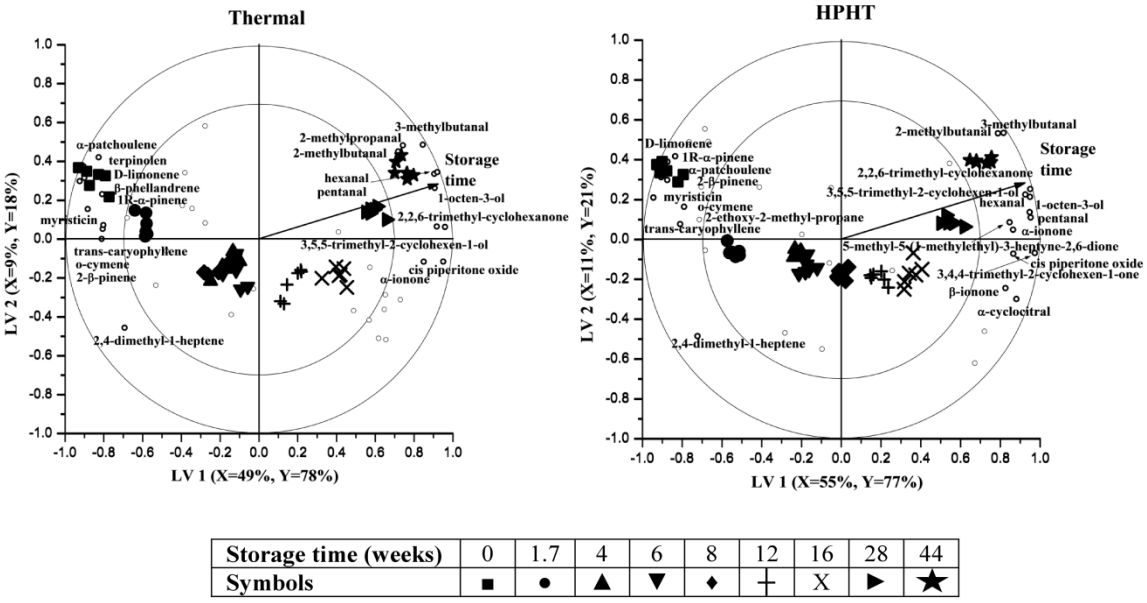


Fig. 2. Total ion chromatogram of the headspace of thermal and HPHT treated carrot puree at the start of storage (day 0), obtained by headspace solid-phase microextraction GC-MS (HS-SPME-GC-MS) fingerprinting. Per chromatogram, the most abundant peaks are identified.

The second trend is the u-shaped structure of the Y-variables (volatile fractions). This shows that there is also a variation in the vertical direction in addition to the horizontal direction. This second variation is described by the second LV. The bi-plots also display the relation between headspace compounds and storage time. Compounds that are located in the same direction as the

Y-vector (the vectors represent the correlation loading for the *Y*-variable (time)) are positively correlated with increasing storage time indicating increase in concentration as a function of storage time, while the ones that are projected in the opposite direction are negatively correlated and decrease with storage time.



Storage time (weeks)	0	1.7	4	6	8	12	16	28	44
Symbols	■	●	▲	▼	♦	┼	X	►	★

Fig. 3. PLS-bi-plots describing the effect of storage on the volatile fraction of carrot puree (objects represented by differently shaped symbols) sterilized with thermal and HPHT processing technologies. The open circles represent headspace components, of which only components selected by the VID procedure are identified and marked in bold (**Table 2**). The vectors represent the correlation loading for the *Y*-variable (time). The percentages of the *X*- and *Y*-variances explained by each latent variable (LV1 and LV2) are indicated on the respective axes.

On the **Figure**, the trend during storage in both thermal and HPHT treated carrot purees is comparable. In addition, on the bi-plots, based on the distance of a component from the center of the coordinate, its importance for displaying the changes during storage can be discussed. For instance, if a compound is projected far from the center and located between the two ellipses (inner and outer ellipses represent correlation coefficients of 70 and 100 %, respectively) on the plot, this shows that the concentration of this compound has largely changed as function of storage time. However, a bi-plot only provides a qualitative graphical representation of the changes in the volatile fraction during storage.

3.1.2. Identification of important shelf-life volatiles and linkage to reaction pathways

To quantitatively rank volatile's importance for the change, VID coefficients were calculated using the selected PLS models (section 2.6.2). Each volatile was assigned with a value between -1 and +1, where a positive VID coefficient represents a higher concentration after storage and *vice versa*. Only those volatiles with absolute value higher than 0.700 were selected to further zoom into and only those were identified (**Table 1; Fig. 3** (bold open circles)). In carrot purees treated with thermal and HPHT processing, respectively, 20 and 22 volatile compounds were selected based on the VID procedure.

Table 1 Volatiles significantly changing as a function of shelf-life, in thermal and HPHT processed carrot purees, selected based on the VID procedure, listed in increasing order of VID coefficient. Positive VID coefficients signify an increase in concentration during storage while negative coefficients denote a decrease. The retention index (RI) is also listed.

PROCESSING	VID	IDENTITY	RI
Thermal	-0.845	β-phellandrene	845
	-0.833	myristicin	1353
	-0.818	D-limonene	899
	-0.810	terpinolen	958
	-0.794	2-β-pinene	847
	-0.778	o-cymene	895
	-0.771	trans-caryophyllene	1262
	-0.770	2,4-dimethyl-1-heptene	680
	-0.745	1R-α-pinene	807
	-0.724	α-patchoulene	1363
	0.794	2-methylbutanal	526
	0.809	α-ionone	1270
	0.825	2-methylpropanal	493
	0.908	cis piperitone oxide	1021
	0.912	3,5,5-trimethyl-2-cyclohexen-1-ol	923
	0.926	3-methylbutanal	521
	0.941	1-octen-3-ol	853
	0.953	2,2,6-trimethyl-cyclohexanone	904
	0.953	pentanal	543
	0.971	hexanal	630
HPHT	-0.874	o-cymene	895
	-0.813	2-β-pinene	847
	-0.812	2,4-dimethyl-1-heptene	680
	-0.786	myristicin	1353
	-0.772	trans-caryophyllene	1262
	-0.769	α-patchoulene	1363
	-0.765	1R-α-pinene	807
	-0.734	2-ethoxy-2-methyl-propane	509
	-0.721	D-limonene	899
	0.751	β-ionone	1322
	0.792	α-cyclocitral	985
	0.828	cis piperitone oxide	1021
	0.845	5-methyl-5-(1-methylethyl)-3-heptyne-2,6-dione	893
	0.853	α-ionone	1270
	0.888	2-methylbutanal	526
	0.915	3-methylbutanal	521
	0.934	3,4,4-trimethyl-2-cyclohexen-1-one	949
	0.953	hexanal	630
	0.953	pentanal	543

0.955	1-octen-3-ol	853
0.973	3,5,5-trimethyl-2-cyclohexen-1-ol	923
0.983	2,2,6-trimethyl-cyclohexanone	904

To increase insight into quality-related shelf-life changes, it was tried to link the significantly changing volatiles to possible reaction pathways. Prior to thermal and HPHT sterilization, the carrot pieces were blanched. In addition, from the microbial analysis, no microbial spoilage was detected during the investigated shelf-life. Hence, enzymatic and microbial activities were not expected to have an impact on the change in volatiles, and changes can be related to chemical reactions. As can be seen from **Table 1**, in both thermally and HPHT sterilized carrot purees, the selected headspace compounds can be categorized into several chemical groups, such as Strecker aldehydes, terpenes, phenylpropanoids, fatty acid derivatives and carotenoid degradation products.

As can be seen from **Table 1**, the Strecker aldehydes (e.g., 2-methylbutanal, 3-methylbutanal and 2-methylpropanal) are selected with a positive VID indicating that the concentrations of these compounds increased in both thermally and HPHT sterilized carrot purees during storage. These volatiles are reaction products of Strecker degradation, one of the side reactions of the Maillard reaction (Cremer & Eichner, 2000a; Kerler, Winkel, Davidek, & Blank, 2010). Although Strecker degradation is a sub-reaction category of the Maillard reaction scheme, it has been described to direct the Maillard reaction towards the aromagenic pathways rather than to chromogenic pathways. In other words, this reaction is of outermost importance in relation to flavor formation (Yaylayan, 2003; van Boekel, 2006; Rizzi, 2008). Strecker aldehydes are often linked to off-flavor development in processed plant-based foods (Cremer & Eichner, 2000b; Yaylayan, 2003; Rizzi, 2008).

From **Table 1**, the concentration of phenylpropanoids such as myristicin and terpenes such as myristicin, D-limonene, o-cymene, 2-β-pinene, trans-caryophyllene, 1R-α-pinene (Kjeldsen,

Christensen, & Edelenbos, 2003; Jones, 2008) seems to decrease during storage in both thermal and HPHT treated carrot purees, which can be due to degradation reactions. In contrast, the concentration of possible fatty acid derivatives such as hexanal, pentanal and 1-octen-3-ol (Reineccius, 2006; Christensen, Edelenbos, & Kreutzmann, 2007) increased during storage. The last group of compounds includes α-ionone, 3,5,5-trimethyl-2-cyclohexen-1-ol and 2,2,6-trimethyl-cyclohexanone in thermally treated carrot purees and β-ionone, α-cyclocitral, α-ionone, 3,4,4-trimethyl-2-cyclohexen-1-one, 3,5,5-trimethyl-2-cyclohexen-1-ol and 2,2,6-trimethyl-cyclohexanone in HPHT treated carrot purees. The formation of these headspace compounds can be linked to carotenoid degradation reactions (Kanasawud & Crouzet, 1990). The concentration of these compounds seems to increase during storage in both thermal and HPHT sterilized carrot purees. Remarkably, the above mentioned important degradation reactions (i.e., terpenes, phenylpropanoids, free fatty acids and carotenoids) are all oxidative-reactions. In our previous studies (Kebede et al., 2013; Kebede et al., 2014a; Kebede et al., 2014b), it was shown that thermal and HPHT sterilization techniques enhanced these degradation reactions immediately after processing. In the present work, it was observed that these oxidative-degradation reactions further continue during storage.

In general, the chemical groups of volatiles significantly changing during shelf-life seem to be comparable in thermal and HPHT sterilized carrot purees. Next, it was investigated whether the reaction kinetics for the changes of these compounds up on storage is different in the differently sterilized carrot purees. Therefore, based on the data from the fingerprinting as a function of time, a kinetic modelling of the volatiles (changing during storage) was performed (see next section).

3.2. Reaction kinetics of the changes of headspace components

Firstly, an appropriate kinetic model was identified for the selected volatile compounds. Next, kinetic parameters, such as reaction rate constants were estimated. As discussed above, during storage, the concentrations of terpenes and phenylpropanoids decreased, whereas the concentrations of Strecker aldehydes, fatty acid derivatives and carotenoid degradation products increased. To show the trend during storage, as an example, the change in the concentration of one compound from each of these chemical groups is presented in **Fig. 4**.

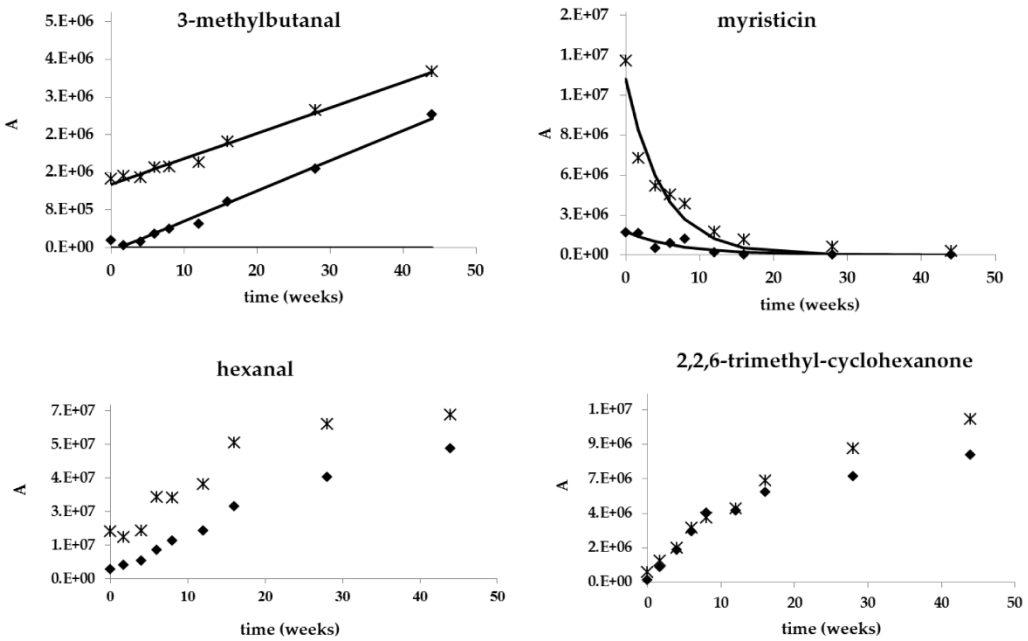


Fig. 4. Peak area (*A*) of 3-methylbutanal (Strecker aldehyde), myristicin (terpene), hexanal (fatty acid derivative) and 2,2,6-trimethyl-cyclohexanone (carotenoid degradation product) as a function of storage time at 20 °C in thermal (♦) and HPHT (x) sterilized carrot purees. The Strecker aldehydes and terpenes could be adequately modelled by a zero-order and first-order kinetic model, respectively.

Immediately after processing, all three Strecker aldehydes (2-methylpropanal, 2-methylbutanal and 3-methylbutanal) were detected at higher levels in thermal sterilized carrot purees compared to HPHT sterilized carrot purees. During storage, the amount of these odor-active volatiles seems to increase in both differently processed carrot purees and this formation could be modelled best by means of a zero-order empirical kinetic model. As can be seen from **Table 2**, the formation

rate during storage was comparable in thermal and HPHT treated carrot purees. One should note that the observed kinetic parameters are empirical, and thus not necessarily reflecting the actual reaction mechanism. They are, however, a useful tool to obtain insight into the impact of storage on compound changes.

In contrast to the Strecker aldehydes, immediately after processing, most of the terpenes and phenylpropanoids were detected at higher levels in HPHT treated carrot purees compared to thermal treated carrot purees. However, up on prolonged storage the concentrations of all terpenes decreased to almost the same level in both differently treated carrot purees. The change in the concentration of phenylpropanoids and terpenes during storage could be modelled best by means of a first-order empirical kinetic model. As can be seen from **Table 2**, for most of the terpenes and phenylpropanoid, the degradation rate was higher in the HPHT treated carrot purees compared to the conventionally treated ones. **Table 3**

Estimated reaction rate constants based on a first-order (for terpenes) and zero-order (for the Strecker aldehydes) kinetic model describing changes in headspace compounds during storage of thermally and HPHT treated carrot purees. Samples were stored at 20 °C up to 44 weeks.

	Thermal processing		HPHT processing	
Compound	*k* (week^{-1})	R^2_{adj}	*k* (week^{-1})	R^2_{adj}
myristicin	0.132 ± 0.042	0.89	0.200 ± 0.031	0.97
D-limonene	0.224 ± 0.036	0.96	0.470 ± 0.102	0.94
terpinolen	0.260 ± 0.037	0.97	0.864 ± 0.152	0.97
2-β-pinene	0.077 ± 0.025	0.92	0.167 ± 0.034	0.95
o-cymene	0.097 ± 0.030	0.91	0.150 ± 0.025	0.97
trans-caryophyllene	0.067 ± 0.026	0.89	0.085 ± 0.016	0.97
1R-α-pinene	0.081 ± 0.027	0.91	0.190 ± 0.049	0.91
α-patchoulene	0.316 ± 0.061	0.95	0.293 ± 0.031	0.98
β-phellandrene	0.183 ± 0.028	0.97	0.106 ± 0.016	0.96
Compound	*k* (area/week)	R^2_{adj}	*k* (area/week)	R^2_{adj}
2-methylbutanal	-60202.4±6359.7	1	-59647.4 ± 3951.1	0.97
2-methylpropanal	-42944.3±2694.9	0.97	-34822.3 ± 3699.2	0.93
3-methylbutanal	-54095.9±2442.6	0.99	-64153.7 ± 3017.7	0.99

As can be seen from **Fig. 4**, during storage, in both thermal and HPHT treated carrot purees, the concentrations of fatty acid derivatives and carotenoid degradation products seem to increase at the beginning of the storage before it reaches a plateau value around week 20. Furthermore, the

amount of most of these compounds starts to decrease by the end of the storage time, possibly due to degradation reactions. Since the changes of these compounds seem to be due to complex formation and degradation reaction pathways, these compounds were not modelled at this stage. In general, the trend during storage of fatty acid derivatives and carotenoid degradation products seems to be comparable in both thermal and HPHT sterilized carrot purees.

4. CONCLUSION

This study clearly showed the potential of the followed integrated *fingerprinting kinetics* approach to increase insight into the evolution of the impact of thermal and HPHT processing during shelf-life. As a case study, carrot puree was selected. The carrot purees were sterilized with thermal and HPHT processing that resulted in equivalent microbial safety (F_0 = 5 min). Differently sterilized carrot purees were stored at ambient storage temperature up to 44 weeks. The volatile fraction of sterilized carrot purees was analyzed with a HS-SPME-GC-MS fingerprinting as a function of storage time (kinetics). In a first step, fingerprinting enabled selection of volatile compounds significantly changing during shelf-life. In both thermal and HPHT sterilized carrot purees, Strecker aldehydes, terpenes, phenylpropanoids, fatty acid derivatives and carotenoid degradation products were selected. The concentrations of terpenes and phenylpropanoids decreased during storage, whereas the rest of the compounds increased. Hence, it can be concluded that the Strecker degradation and oxidation-related degradation reactions (such as terpenes, phenylpropanoid, fatty acid and carotenoid) seem to take place during storage of thermal and HPHT treated samples. In a second step, based on the data from the fingerprinting as a function of storage time, kinetic modelling of the change in selected volatiles during ambient storage was performed. A zero-order and first-order empirical kinetic model could adequately model the changes in Strecker aldehydes and terpenes and phenylpropanoids up on storage, respectively. One should, however, realize that the obtained

kinetic parameters pertain to elementary reactions and care should be taken when interpreting these parameters. Nevertheless, the applied simple empirical kinetic models are proven to be useful tools to obtain insight into the changes of the selected volatile compounds during storage. Even though the Strecker aldehydes were detected at higher levels in thermal sterilized carrot purees immediately after processing, during storage, the compounds increased at a comparable rate in both the differently processed carrot purees. Therefore, it can be stated that the difference in the final concentration of the Strecker aldehydes between thermal and HPHT sterilized carrot purees is dependent on the applied preservation techniques. In contrast, immediately after processing, most of the terpenes and phenylpropanoid were detected at higher levels in HPHT treated carrot purees compared to the conventionally treated ones. During storage, however, the degradation rate was faster in HPHT treated carrot purees compared to the conventional treated samples. Consequently, the concentrations of all terpenes and phenylpropanoids decreased to almost the same level in both the differently treated carrot purees by the end of storage. Therefore, even though HPHT processing seem to preserve most of the naturally existing terpenes and phenylpropanoids immediately after processing, after prolonged ambient storage, both processing technologies seems to have a comparable impact. With respect to fatty acid derivatives and carotenoid degradation products, in general, the trends during storage seem to be comparable in both thermal and HPHT sterilized carrot purees.

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7. List of figure captions

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